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Contraceptive and Abortificent Effects of Aqueous Crude Extract of Sclerotia of *Pleurotus tuberregium* on Non pregnant and Pregnant Wistar Rats

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ABSTRACT

Mushrooms have been used in many folk medicine traditions to treat a wide range of ailments including infertility. Researchers have demonstrated a broad array of therapeutically significant compounds in mushrooms species which are currently believed to be in use in mainstream medicine. In the south-south region of Nigeria there have been reports from pregnant women who used the sclerotia of Pleurotus tuberregium during early pregnancy and discovered that it caused abortion, thus the need to scientifically study the effect of sclerotia of P. tuberregium on pregnant albino wistar rats. The effect of the aqueous crude extract of P. tuberregium on some fertility parameters was evaluated on pregnant female albino wistar rats to determine the safety of the sclerotia of P. tuberregium in pregnancy. In the course of the experiment acute toxicity study of the extract was first carried out on female albino mice and doses were selected. The aqueous crude extract of P. tuberregium was administered to two groups of twenty (20) female rats each. The first group of rats was mated and after seven days of pregnancy the rats were administered oral doses of 100, 500, and 1000 mg/kg per day for 7 days after which they were sacrificed on the 14th day of pregnancy. The second group of rats was mated and administered oral doses of 100, 500, and 1000 mg/kg per day for 21 days and sacrificed. The results revealed that the extract was void of the causing death to any of the mice. The reproductive parameters (progesterone significantly increased while oestrogen, follicle stimulating hormones and luteinizing hormones) significantly decreased in tested animals within the treated period compared to the control. The pregnancy outcome results revealed there was no foetus in the uterus of the supposed pregnant rats compared with the control. The results from this experiment revealed that the extract has contraceptive and abortificent effects. This study therefore suggests that the sclerotia of P. tuberregium is useful as a contraceptive drug and can be recommended for medical use by women that are not desirous to have children.

1. Introduction

Pleurotus tuberregium popularly referred to as “king tuber mushroom”, is an edible gilled fungus native to the tropics including Africa. It is a saprotroph found on dead wood, including Daniela trees in Africa [1]. As the fungi consume the wood, it produces a sclerotium (storage tuber), either within the decaying wood or in the underlying soil. These sclerotia are up to 30 cm wide, round and dark brown with white interiors. The fruit bodies then emerge from the sclerotium. However, the sclerotium and the fruit bodies are both edible. *P. tuberregium* is also nematophagous, caching

nematodes by paralyzing them with a toxin [2]. Mushrooms possess 92 % water, 4 % carbohydrate, 2 % protein and less than 1 % fat. Some extracts from mushroom are possible treatment of various diseases such as cancer, though might not be scientifically confirmed [3]. Some mushrooms are useful as fertility drugs while others are used as antifertility drugs regulating progesterone and oestrogens hormones in females. Progesterone is occasionally referred to as the "hormone of pregnancy" and has many roles relating to the development of fetus by; converting endometrium to its secretory stage to prepare the uterus for implantation. At the same time progesterone affects the vaginal epithelium and cervical mucus, making it thick and impenetrable to sperm [4]. The metabolic effects of oestrogen in postmenopausal women have been linked to the genetic polymorphism of ER. Though Oestrogen is present in male and female, they are usually present at significant higher levels in females of reproductive age [5]. They promote the development of female secondary sexual characteristics such as breast, and are also involved in the thickening of the endometrium and other aspects of regulating the menstrual cycle. In males, oestrogen regulates certain functions of the reproductive system important to the maturation of sperm and may be necessary for a healthy libido. For these reason oestrogen and progesterone were selected for the female fertility study of Sclerotia of *P. tuberregium* on pregnant and non-pregnant albino wistar rats.

2. Methodology

2.1. Location of study

This study was carried out in the animal house of the Department of Pharmacology/ Toxicology, Faculty of Pharmacy, University of Benin, Benin city, Edo State. Nigeria.

2.2. Preparation of sample

The sclerotia of *Pleurotus tuberregium* used in this study were obtained from Oliha market, located in Siloku road, in Oredo local government area, Benin City, Edo State, Nigeria. The sclerotia of *P. tuberregium* was identified and confirmed by Dr. Abbot Oghenekaro a mycologist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

2.3. Experimental Animals

Adult male rats with proved fertility and sexually naive female wistar albino rats of average weight 180- 200 g, were obtained from the Animal house in the Department of Pharmacology/ Toxicology, Faculty of Pharmacy, University of Benin. The rats were housed in the animal house and kept in cages with ambient temperature and maintained under standard laboratory conditions, which included 12-hour light and 12-hour dark cycle. The rats were separated into two groups of twenty (20) female rats each and were fed with standard diet and clean water *ad libitum* for seven days as acclimatization period prior to the experiment. Experimental animals were deprived of food overnight before the study and were handled in accordance with the organization for economic and co-operation and development (OECD) 420 guideline.

2.4. Preparation of extract

Fresh sclerotia were air dried for five days after which the brownish rough back was peeled off with a knife to expose the whitish inner part and thereafter grinded into powdery form.

2.5. Extraction of Material

One kilogram (1 kg) of the powdered sclerotia material was extracted with distilled water using kedjahl heating mantle. The resulting crude aqueous extract was then filtered by passing through a

cheese cloth which was concentrated in a water bath and finally dried in an oven at a temperature of 45 ^{0} C for 48 hours.

2.6. Mating Procedures

Sexually naive female rats were mated with male rats of proven fertility in a ratio of 1:1. Only females at pro-estrus after microscopic examination were mated. Mated female rats were left overnight with their male canter part. Appearance of sperm in the virginal the next morning by microscopic examination was taken as day zero of pregnancy.

2.7. Pregnancy out comes

All animals in each group had uterine horns examined for implantation and resumption sites, were compared with the control. Animals were examined daily for any sign of toxicity such as bleeding, diarrheal, salivation, tremors, writhing, convulsions, hair loss, behavioural abnormalities, and or mortality. On the 14th day of gestation (7th day of treatment), the female rats were laparotomized under chloroform anaesthesia. The lower abdomen was cut open; uterus was examined for pregnancy outcome, the presence of resorption sites and live or dead foetuses.

2.8. Experimental design

2.8.1. Acute toxicity study

20 mice weighing 30-35 g were divided into four (4) groups of five (5) mice each were administered 2000, 1000, 500 and 100 mg/kg per body weight of the extract orally for 14 days and observed for mortality and signs of behavioural and neurological toxicity.

2.8.2 Abortificent and contraceptive studies

Forty female rats were grouped into two broad groups of twenty female rats each. The first group was used for abortificent study while the second group was used to study the contraceptive effect of crude aqueous extract of sclerotia of *pleurotus tuberregium.* The first group was made up of 20 female (pregnant) rats, re-grouped into four sub-groups of 5 rats each and were mated. After seven (7) days gestation, rats were administered; distilled water, 100, 500, and 1000 mg/kg of crude aqueous extract of sclerotia of *P. tuberregium* daily for seven days. The second group also made up of 20 female (non- pregnant) rats were mated and alongside administered 100, 500 and 1000 mg/kg of crude aqueous extract of sclerotia of *P. tuberregium* daily for 21 days.

Group 1 (normal control) - received distilled water
Group 2- received 100 mg/kg of extract
Group 3- received 500 mg/kg of extract
Group 4- received 1000 mg/kg of extract

The animals were sacrificed after the period of treatment. The bloods of animals were collected by vein puncture with the use of sterilized syringe and were transferred into specimen bottles containing anti-coagulant and plasma separated by centrifuging the blood at 3000 revolutions/min. and stored at -20 ^{0}C.

Results were expressed as mean ± SEM (standard error of mean). Data were compared using one-way analysis of variance (ANOVA). Microsoft excels and Graph pad prism 6 version software (UK) was used for all data analysis. $P < 0.05$ was regarded as indicating significant difference.

3. Results and Discussion

3.1. Acute toxicity study

P. tuberregium was evaluated for acute toxicity at doses of 2000 mg/kg, p.o. The extract was revealed to be safe since there was no mortality of any mice. Hence, the doses selected for the abortificent and contraceptive activity were 100, 500 and 1000 mg/kg, p.o. Table 1 shows the effect of aqueous extract of the sclerotia of *P. tuberregium* on the pregnancy outcome of pregnant female rats.

Table1: Aqueous extract of the sclerotia of *P. tuberregium* on the pregnancy outcome of pregnant female rats.

Treatment groups (mg/kg)	Quantal pregnancy (%)	No. of uterine resorption sites	Resorption index (%)	Pre-implantation	Pre-implantation loss (%)
Distilled H_20	100	0	0	10	0
100	100	10	100	10	100
500	100	8	80	10	80
1000	100	7	90	09	90

The female rats were administered 100, 500 and 1000 mg/kg/day extract at 14th day of pregnancy (mid pregnancy). Data are expressed as means ±SEM compared to control, n= 5 in all treated groups.

The results obtained from this experiment showed that pregnant rats treated with aqueous extract of the sclerotia of *pleurotus tuberregium* exhibited abortificent activities. The number of resorption site was 100, 80 and 90 % for 100, 500 and 1000 mg/kg aqueous extract of the sclerotia of *pleurotus tuberregium.* This finding is in line with [6] who reported the abortificent efficacy of *Rumex steudelli* (Tult) root on pregnant rats.

3.2 Hematological investigation

Table 2 shows the summary of the results obtained from hematological analysis of blood samples collected from the dams.

Table 2: Hematological analysis

Parameters	Control	100 mg/kg	500 mg/kg	1000 mg/kg
WBC 10^3 µl	10.2 ± 0.6	6.5 ±0.5 ^{c}	5.6±0.5 ^{c}	3.8 ±0.5 ^{d}
PCV %	41.6 ± 0.9	40.4 ± 1.2	42.0 ± 0.9	47.8± 0.7^b
RBC 10^3 µl	7.4 ±0.3	8.2 ±0.2	9.5 ±0.2 ^{c}	9.7 ±0.3 ^{d}
Plat 10^3 µl	516 ±16.4	343 ±17.4 ^{d}	476 ±15.0	527 ±18.7
Hb g/dL	14.8 ±1.8	16.1 ±2.5	17.1 ±1.5	19.2 ±1.7

Lymp %	90 ±3.5	87 ±3.7	74 ±1.7	64 ±4.2 [d]
Mono %	3.8 ±0.5	2.6 ±0.3	9.3 ±0.3 [d]	14.2 ±0.9 [d]
Eosin %	1.6 ±0.3	1.7 ±0.4	2.2 ±0.5	1.3 ±0.2

Values are expressed in mean ± SEM as compared with control

Key: WBC White blood Cell PCV Packed cell volume
RBC Red blood Cell Plat platelet count
Hb Haemoglobin Lymp lymphocytes
Mono Monocytes Eosin Eosinophil

The hematological study revealed that the aqueous extract of the sclerotia of *P. tuberregium* reduced White Blood Cell in all treated groups. A significant decrease ((P<0.001) in Platelet count was observed in animals treated with 100 mg/kg body weight of the extract. While no significant change was observed in the other treatment groups. A significant decrease ((P<0.001) in lymphocyte counts was observed in animals treated with 1000 mg/kg body weight of the extract. While no significant change was observed in the other treatment groups. This suggests that the changes in the effect of this extract may be an attribute that it contains some bioactive constituents which produces glycoside. A significant increase in PCV was observed in animals treated with 1000 mg/kg body weight of the extract. While no significant change was observed in the other treatment groups. A significant increase in Monocytes count (P<0.001) was observed in animals treated with 500 and 1000 mg/kg body weight of the extract. A significant increase in and RBC (P<0.01, P<0.001) was also observed in animals treated with 500 and 1000 mg/kg body weight of the extract There were increases in Hb in animals treated with 100, 200 and 1000 mg/kg of extract although not significant. This finding is in line with [7] who recorded an increase in hematological studies in their experiment using methanolic extract of *moringa oleifera* on rats.

Body Weight Index:

Results obtained from the body weight index of the dams during the course of pregnancy, revealed that even in the extract treated groups there was initially increase in weight from day 0-7 but this declined from day 8-14 after bleeding was observed 24 hours after administration. Dams had successful progression of pregnancy and development from day 0-7 but declined from day 8-14 (Table 3).

Table 3. Body weights index of dams from day 0 to 14th day of pregnancy.

Treatment groups (mg/kg)	Day 0	Day 7	Day 14
Control	183.38±0.1	213.9±0.2	224.2±0.3
100	184.8±3.3	218.1±3.9	185.7±2.4^{d}
500	180.8±1.3	204.2±5.2	175.9±4.1^{d}
1000	183±3.5	206.6±5.2	187.6±2.6^{c}

Key: [c] p< (0.001), and [d] p< (0.0001).
On the abortificent study the report showed that the extract at all the treated groups exhibited abortificent activity in pregnant rats. Administration of the extract caused partial bleeding which was seen early in the morning 24 hours after oral administration of the extract thus leading to adverse effect on the fetal development in pregnant rats. This indicated abortion which was confirmed by reduction in the weights of dams. [8] reported that the presence of saponins, tannins, steroids, alkaloids, glycosides and Terpenes in *Aspilia Africana* was found to possess abortificent activities.

Table 4: Hormonal change connected with daily administration of extract to mature non pregnant female rats.

Hormone	Control	100 mg/kg	500 mg/kg	1000 mg/kg
Progesterone (10^3 µl)	0.110±0.2	0.310±0.2	0.432±0.2	0.503±0.4 [a]
Follicle stimulating hormone (mlu/m)	4.18±0.2	3.69±0.1 [a]	2.70±0.1 [b]	1.20±0.1 [c]
Leuthenizing hormone (mlu/m)	4.67±0.2	3.54±0.1 [a]	3.8±0.6 [a]	2.4[d]±0.1 [d]
Oestrogen hormone Pg/ml	9.07±0.1	8.60±1.9 [a]	7.2±1.6 [c]	6.9±0.3 [c]

Values are mean ± SEM. n = 5 [a] P<0.05 in comparison with the control group. [b] P < 0.01 [c] P < 0.001. [d] P< 0.0001.

All animals studied for contraceptive activity showed increase and decrease in progesterone and oestrogen levels with 1000 mg/kg being the highest and lowest respectively when compared to control. This is in line with the work of [9] who reported that ethanolic and aqueous extracts of *Calotropis procera* roots interrupted the normal oestrous cycle in 60 and 80 % of treated rats respectively. It was reported by [10] that the ethanol extract of *Rivea hypocrateriformis* was administered orally at the dose level of 200 and 400 mg/kg body weight to adult albino rats and resulted in an irregular oestrous cycle with shortened oestrous and metestrus with extended proestus in non-dose dependent manner and increased in the weight of the uterus, its thickness and diameter indicating the uterotrophic effect of the extract. Report by [11] indicated that the oestrous cycle of rats became irregular with prolonged oestrous and metestrous phases and reduced di-estrous and pro-eostrous phases after oral *Momordica charantia* at a dose level of 25 mg/100 g/ body weight. The results showed reduced ovarian weight, the result showed irregular oestrous cycle with shortened oestrous and metestrus with extended pro-eostrous in non-dose dependent manner and increased in the weight of the uterus.
Progesterone is anti- mitogenic in endometrial epithelia cells and as such mitigates the tropic effect of oestrogen. If pregnancy does not occur, progesterone level will decrease, leading to menstruation. Normal menstrual bleeding is progesterone-withdrawal bleeding. If ovulation does not occur and corpus lutetium does not develop, levels of progesterone may be low leading to an

ovulatory dysfunction uterine bleeding [12]. During implantation and gestation, progesterone decreases maternal immune response to allow for the acceptance of the pregnancy. Progesterone reduces contractility of the uterine smooth muscle. Progesterone also inhibits lactation during pregnancy. The fall in progesterone levels following delivery is one of the triggers for milk production [13]. A drop-in progesterone levels is probable one of the steps that facilitates the onset of labor. The feotus metabolizes placenta progesterone in the production of adrenal steroids. Oestrogen on the other hand is the primary female sex hormone as well as pills. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. Oestrogen may also refer to any substance natural or synthetic that mimics the effect of the natural hormone [14]. The estran steroid estradiol is the most potent and prevalent endogenous oestrogen even though several metabolites of estradiol have estrogenic hormonal activity. Oestrogens are used medically as part of some oral contraceptives, in hormone replacement therapy for postmenopausal, hypogonadal and transgender women and also in certain hormone-sensitive cancers like prostrate and breast cancers. There are one of three types of sex hormones the others are androgens/anabolic steroids like testosterone and progestogen such as progesterone [15]. Oestrogens are synthesized in all vertebrates and some insects. Their presence in both vertebrates and insects suggest that oestrogenic sex hormones have an ancient evolutionary history. The three majors naturally occurring forms of oestrogen in women are estrone (E1), estradiol (E2) and estriol (E3). Another type of oestrogen called sterol (E4) is produced only during pregnancy. Quantitatively, oestrogen circulates at lower levels than androgens in both men and women [16]. While oestrogen levels are significantly lower in males compare to females, oestrogen nevertheless has important physiological roles in males. Like all steroid hormones, oestrogen readily diffuses across the cell membrane. Once inside the cell, they bind to and active oestrogen receptors (ERs) which in turn modulate the expression of many genes. Additionally, oestrogens bind to and activate rapid- signaling membrane oestrogen receptors (mERs) [9]. The actions of oestrogen are mediated by the oestrogen receptor (ER). Like other steroid hormones, oestrogen enters passively into the cell where it binds to and activates the oestrogen receptor. The oestrogen: ER complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes (in a study using an oestrogen- dependent breast cancer cell line as model, 89 such genes were identified) [17]. Since oestrogen enters all cells, its actions are dependent on the presence of the ER in the cell. The ER is expressed in specific tissues including the ovary, uterus and breast. Furthermore, there are several other structural changes induced by oestrogen in addition to other functions [14]. Oestrogens are responsible for maturation and maintenance of the vagina and uterus and are also involved in ovarian function, such as maturation of ovarian follicles. In addition, oestrogens play vital role in the regulation of gonadotropic secretion.

The contraceptive index in all the treated groups was 100 % as none of the female rats mated and treated with the extract were pregnant after 21 days treatment compared with the control group which littered on that day. These results are in line with those of [18] who reported 90 % contraceptive activity of *Acalpphs indica* extract. In the same vein, [5] reported that 29 animals out of 30 showed contraceptive activity of *Mentha arvensis* stem bark. Researchers have attributed the mechanism behind the contraceptive activity of herbal remedies to chemical compounds contained in them. These include alkaloid, phenols, steroids, reducing sugars, triterpenoids, tannins, and free amino acids. Although the present study did not carry out the phytochemical analysis on *pleurotus tuberregium* extract, [19] reported that these chemical compounds carry out their contraceptive activities by suppressing the release of gonadotropins, inhibiting follicular development and preventing ovulation as its primary mechanism of action.

Over population has become a problem worldwide especially in Africa. It is therefore important to discover remedies using biological means with reference to modulation in the human fertility ability due to the side effects of contraceptive orthodox drugs [9]. Epidemiological studies have

shown that oral hormonal contraceptives are inflating risk of cerebral thrombosis, increase in serum triglyceride and cholesterol, causes malignant tumors, headache, diabetes, abdominal pain, nausea and menstrual changes [4]. Most of these contraceptives contain oxynol-9 a potential spermicidal agent which has been shown to cause inflammation and ulceration of the genital organ, it is also a risk factor of HIV-1 infection when used repeatedly [20]. These and many other reasons have necessitated formulations of new herbal remedies with anti- spermatogenic activity.

4. Conclusion

Medicinal mushrooms have been used since ancient times by different people who use them in various ways as source of medicine. From this valuable animal study, it was revealed that this mushroom (*Pleurotus tuberregium)* has significant abortificent and contraceptive activities. Therefore, *P. tuberregium* is an antifertility agent, it should be avoided in pregnancy and in women desirous to conceive.

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